Solid-Surface DNA Sequencing Array

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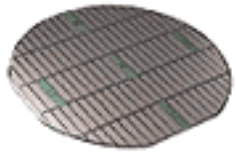

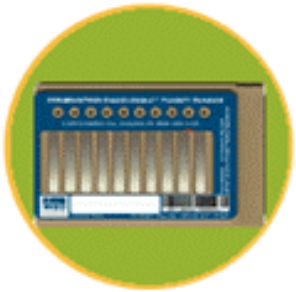

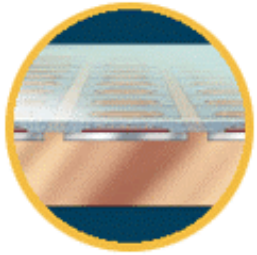

Description

The objective of this proposal is to develop a solid surface chip that can sequence up to 100,000 different molecules of nucleic acid, DNA or RNA, without the use of cloning, or conventional electrophoresis and modified nucleosides. This will be accomplished with the use of high-density oligonucleotide arrays and Pyrosequencing method. In this sequencing-by-synthesis method, a cascade of enzymatic reactions yields detectable light, which is proportional to incorporated nucleotides. Pyrosequencing has the advantages of accuracy, flexibility and parallel processing that can be achieved on the surface of a high-density oligonucleotide array.

Innovative Claims/NASA Significance

- Novelty lies in the use of specialized oligonucleotide arrays that may serve as primers on DNA sequencing templates. Through the use of pyrosequencing one can determine the base at any position in the DNA since the liberation of PPi during the incorporation of nucleotides into the extension reaction generates light through subsequent enzymatic processes.
- Parallel nature of data acquisition inherent in the proposed method make it attractive since it has the potential to increase the throughput of DNA sequence data generation significantly.

Plans

•Determine the best oligonucleotide sequences for hybridization, using computational approach.
•Demonstrate ability to detect specific DNA fragments from a mixture of genomic DNA by hybridization.
•Demonstrate quantitative synthesis of DNA from genomic DNA using polymerase enzymes.
•Test the solid-surface oligonucleotide array to determine the best length of oligonucleotides for synthesis of DNA by the use of polymerase enzymes.
•Demonstrate ability to distinguish between sequence-specific DNA synthesis by the use of polymerase enzymes.
•Determine feasibility of detecting and analyzing single base mismatch between template genomic DNA and array-immobilized oligonucleotide.
•Demonstrate ability to sequence short strands of DNA (approximately 1 to 30 nucleotides).
•Develop methods to detect single nucleotide polymorphism.
•Develop prototype DNA/RNA sequencing device.